A CRITICAL CONSIDERATION OF c-MITOSIS WITH REFERENCE TO THE EFFECTS OF NITROPHENOLS.

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(Plate xiii.)

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Synopsis.

Nitrophenols, as well as many other substances, suppress spindle formation and for this reason have been described as c-mitotic. Many authors either assume without justification that substances which suppress spindle formation have a common mode of action, or do not recognize that, by their use of this term, they imply a common mode of action for all such substances.

Although nitrophenol and colchicine cause spindle suppression, evidence is presented which indicates that their modes of action are probably different. It is essential that the term "c-mitotic" be confined to substances which have true colchicine action. If this distinction is not observed, further confusion must result, which will tend to hamper research on mitotic poisons.

In 1938 Levan described the cytological action of colchicine on plant cells. He showed that colchicine suppressed spindle formation and induced polyploidy without apparently affecting any other cell process. The name "c-mitosis" was given to this phenomenon. Since that time the cytological action of a large number of chemicals has been investigated and there are reports of numerous substitutes for colchicine. Among substances reported to have an action like that of colchicine are phenols and quinones (Levan and Tjio, 1948), veratrine sulphate (Witkus and Berger, 1944), cryptopleurine (Barnard, 1949), sulphonamides (Peters, 1946; Fuller, 1947) and various inorganic salts (Levan, 1945; Galinsky, 1949). Although these compounds are chemically unrelated, the fact that they are reported to cause a similar cytological effect suggests the possibility of a common mode of action. It seems reasonable to suppose that stages in cell division may be linked with metabolic processes and that cytological aberrations may well result from artificial disturbances in the metabolism of the cell. There are many substances which are known to interfere with physiological processes in the cell, and the cytological action of some of these may be of interest.

Recent workers on the phosphorylation mechanisms have made great use of 2,4-dinitrophenol, which seems to be a specific inhibitor of the phosphate transfer mechanism in both plant and animal cells. The mononitrophenols do not have this specific effect (Cross *et al.*, 1949). The mononitrophenols are reported to cause c-mitosis (Levan and Tjio, 1948*a*, 1948*b*). This would suggest that c-mitosis is not the result of interference with the phosphate transfer mechanism. In view of the importance of such a conclusion, it seemed desirable to reinvestigate the c-mitotic activity of nitrophenols.

During the course of this investigation it became apparent that there is a marked difference between the cytological abnormalities induced by colchicine and phenols. The cytological action of phenols does not seem to be "c-mitosis" and this may well apply to many of the substances which have been described as "c-mitotic". If this is so, it is essential to distinguish between true colchicine mitosis and other abnormal types of mitoses before attempting either to look for a common mode of action or even to evaluate the enormous literature on mitotic poisons.

Recovery experiments are an essential part of this type of investigation, as it is necessary to know whether the roots are alive at the end of the experiment and also whether induced cytological abnormalities are permanent or only temporary changes.

In the experiments to be described, onion bulbs with roots about 3 cm. long were transferred from tap-water to the test solutions for periods of 1, 2, 4, 8, 12, 24 and 48 hours. Solutions of dinitrophenol, o- and p-nitrophenol at concentrations 3, 30 and 300 mg/l were made up in tap-water. After treatment some roots were fixed in acetic-alcohol (3:1) and examined by standard aceto-orcein and Feulgen squash methods (Darlington and La Cour, 1947). The remaining roots were transferred back to tap-water for periods up to 48 hours, and recovery estimated by the ability of the roots to continue or resume growth. At intervals during recovery roots were fixed for examination.

TABLE 1.

Time of Treatment.	2, 4-dinitrophenol.			p-nitrophenol.		
	3 mg/l.	30 mg/l.	300 mg/l.	3 mg/l.	30 mg/l.	300 mg/l.
1 hour.	Metaphase chromosomes short and thick.	Spindle suppressed.	Spindle suppressed. Stickiness at anaphase. Resting nuclei granular.	Normal.	Metaphase chromosomes short and thick in few cells.	Spindle suppressed
2 hours.	Metaphase chromosomes short and thick. Spindle suppressed in a few cells.	Spindle suppressed. Stickiness at anaphase.	Spindle suppressed. Stickiness at anaphase and telophase. Resting nuclei granular.	Few metaphase with short, thick chromosomes.	Metaphase chromosomes short and thick.	Spindle suppressed. Stickiness at anaphase.
4 hours.	Metaphase chromosomes short and thick. Spindle suppressed in more cells.	As 2 hours.	As 2 hours.	As 2 hours.	Metaphase chromosomes short and thick. Spindle suppressed in some cells.	Spindle suppressed. Stickiness at anaphase and telophase. Resting nuclei slightly granular.
24 hours.	Spindle suppressed. Anaphase and telophase rarely found.	As 2 hours, but resting nuclei affected.	As 2 hours, but all cells becoming diffuse.	Metaphase chromosomes short and thick. Spindle suppressed in few cells.	Spindle suppressed. Resting nuclei slightly affected.	As 4 hours, but resting nuclei more granular.
In water after treatment.	All recovered.	Recovery after 2, 4, 8 hours. Not after 24 hours.	No recovery.	All recovered.	All recovered.	No recovery after 24 hours.

Cytological abnormalities induced by nitrophenols varied with the concentration, length of time of treatment, and division stages of the cells at the beginning of treatment. Cytological observations mentioned below are those obtained using dinitrophenol, but the effects with p- and o-nitrophenol were the same except that 2,4-dinitrophenol is effective at lower concentrations. This concentration difference is shown in Table 1.

At the lowest concentration abnormalities were observed after two hours. Metaphase and anaphase chromosomes were short and thick, but otherwise the mitotic figures were normal. After four hours most of the cells in metaphase developed a normal metaphase plate with short thick chromosomes, but a few cells had chromosomes scattered at random in the cytoplasm. Twenty-four hours' treatment allowed most cells

dividing during treatment to proceed to normal resting cells; some cells remained in "blocked" metaphase, but unlike colchicine-treated cells, there was no evidence of centromere division. These observations indicate that in the presence of nitrophenols at low concentration spindle formation is completely suppressed, at least in some cells.

At higher concentrations the effects of dinitrophenol were noticeable in one hour. In some cells a metaphase plate appeared in a normal manner; in others short thick chromosomes were scattered in the cytoplasm or clumped together in the centre of the cell. Anaphase stages were numerous; some were normal, some had lagging chromosomes, and some had short thick chromosomes. This variability in the behaviour of different nuclei could be due to irregular penetration across the root in a short period of time. More than two hours' treatment at 30 mg/l induced stickiness in anaphase chromosomes, with sticky bridges in anaphase and telophase stages.

Changes in the resting nuclei were observed after 48–60 hours at 3 mg/l, after 24 hours at 30 mg/l and after 2 hours at 300 mg/l. Some resting nuclei lost their fine-grained appearance, and the chromatin coagulated into coarsely grained masses, forming nuclei about the same size as healthy resting nuclei. Others appeared as small deeply stained "pycnotic" masses with nucleoli standing out as unstained spots. With longer treatment at the high concentrations resting nuclei and chromosomes became diffuse and unstainable, and even cell walls were indistinct.

Length of treatment at any one concentration influenced the ability of the roots to recover. When roots which had been in 3 mg/l for 1–24 hours were transferred to tap-water, all roots recovered, but after 24 hours in 30 mg/l or 1 hour in 300 mg/l no recovery was observed. These roots became soft and flaccid at the tip.

The cells of roots which recovered in tap-water following treatment all commenced normal division after varying periods of time. Again there is a relationship between length of treatment and concentration. Resumption of normal division is rapid after treatment at low concentrations, but much slower after high concentrations. When transferred to water, all dividing cells pass into the resting state and there is a delay depending on length of treatment at each concentration before division begins. As all the cells which resume division are normal and diploid, it is possible that only cells which did not divide during treatment are seen in mitotic stages after recovery.

If resting nuclei were affected, the roots did not recover when the nitrophenol was washed out. Roots which had been in 300 mg/l even for one hour did not have normal dividing cells after being in water for periods up to 48 hours. Abnormal division stages exactly like those of treated roots were observed in these flaccid roots. These results of recovery experiments, particularly those following treatment with 300 mg/l, showed that nitrophenols possess a strong toxic action. At concentrations of 30 and 300 mg/l, nitrophenols appeared to behave as poor fixatives gradually killing and fixing the cells in the stage in which they were when the substance penetrated the cells.

The cytological abnormalities induced by mono-nitrophenols on meristematic cells has been described by Levan and Tjio (1948) as "c-mitosis" over a limited concentration range. This term "c-mitosis" was originally used by Levan (1938) as follows. "The effect of colchicine is entirely specific and the modification in mitotic behaviour will be abbreviated 'c-mitosis'. The c-mitosis can be referred to one single moment, viz. an inactivation of the spindle apparatus connected with a delay of the division of the centromere. The effect thus produced may be expressed as a completion of the chromosome mitosis without nuclear or cellular mitosis."

Levan then discusses the "course of c-mitosis", referring the term not to "one single moment", but to the whole abnormal cell division process which results in telophase nuclei with twice the original number of chromosomes, and numerous polyploid cells in the meristem. This explanation of the term "c-mitosis" is unfortunately ambiguous. The first part of the explanation refers to the specific action of colchicine on the spindle mechanism, whereas the second part, and the subsequent discussion, refers to the whole cell division process. Since 1938 both the spindle

inhibition and the morphological picture of the colchicine mitosis have been described as "c-mitosis" by Levan and other workers. It seems necessary to make a distinction between these two aspects, since the work with nitrophenols indicates that spindle suppression is not necessarily the same as colchicine action.

It seems generally accepted that colchicine has the single effect of upsetting the spindle mechanism, thus altering metaphase and consequently anaphase separation and cell wall formation. Other cell division processes, including the stages from resting nucleus to metaphase and also centromere division, are allowed to proceed during colchicine treatment and the result is polyploidy.

As shown earlier (pages 159–160), nitrophenols are not "entirely specific" in their action. They cause inactivation of the spindle mechanism, but they also alter other vital and independent cell processes so that centromere division, that is "completion of the chromosome mitosis", rarely takes place in treated cells. A very small percentage of cells became tetraploid during treatment, probably only those beginning metaphase or anaphase at the time of treatment, but no tetraploid cells were seen in recovered roots. Therefore, polyploidy, the permanent consequence of "c-mitosis", was not observed as a result of nitrophenol treatment.

As nitrophenol-treated roots continue to grow by normal cell division when they recover, it is not possible to determine whether the cells which show abnormalities during treatment recommence mitosis on recovery. The normal diploid cells may come from the large number of cells which remain in resting stage during treatment and these may completely outgrow abnormal cells. Roots did not recover when the resting nuclei were visibly affected, and this may indicate either that these are the cells which continue growth or that granulation of the resting nuclei occurs only when all the cells are killed.

Attempts to explain the action of colchicine on cell division, or to correlate the physical and chemical properties of colchicine with those of other spindle suppressors so far have been unsuccessful. The explanation of this would seem to be that colchicine has a specific action on the spindle not possessed by other spindle suppressors. It has been suggested (Levan and Ostergren, 1943; Ostergren and Levan, 1943; Ostergren, 1944) that there is a negative correlation between water solubility and the ability of numerous substances to suppress the spindle. Colchicine, however, has a very high water solubility and a strong activity, and does not fit this general theory. This exception is too important to be disregarded and it must be assumed in contrast to the assumption of Ostergren (1944) that colchicine activity is not the same as the spindle suppression of other substances.

During division it is the spindle which is most sensitive to disturbance and there are many observations to show that it is easily upset by changes in the environment of the cell, e.g. temperature changes (Barber and Callan, 1943). Spindle formation in plant and animal cells almost certainly depends on a number of reactions in the cell and upset of one or more of these reactions may produce the same results at metaphase. If the term "c-mitotic" is to be used for any spindle inhibiting substance, it can be applied to a wide variety of substances which probably act on different parts of the spindle mechanism. On the other hand, if it is to be used for true colchicine-like action, it applies only to a particular action on one part of the cell division process. It would be better to retain the term "c-mitosis" for substances which are known to have the same specific action on the spindle mechanism as has colchicine and no other activity. This view agrees with that of Frahm-Leliveld (1949), who, after testing many substances, concluded that only colchicine and acenaphthene act as polyploidogenic agents on plant cells and are the only known "c-mitotic" substances.

Levan (1938) has shown that 2% colchicine for 72 hours produced cytological abnormalities but was not toxic to the cells. With high concentrations the toxic action of the nitrophenols is clearly demonstrated by recovery experiments. Treated cells which did not recover, retained their abnormal cytological figures until all cell contents became diffuse and unstainable, indicating that vital cell processes were stopped.

Concentrations of mononitrophenols reported by Levan and Tjio (1948) to induce "c-mitosis" were 5×10^{-2} to 2×10^{-3}M for o-nitrophenol, 1×10^{-2} to 1×10^{-3}M for m-nitrophenol and 2×10^{-2} to 2×10^{-4}M for p-nitrophenol. All solutions were diluted with distilled water. These concentrations are higher than those used in our experiments where $2{\cdot}16 \times 10^{-3}$M, the highest concentration, killed in less than 24 hours. The toxic action of phenols is very much greater in distilled water than tap-water, probably due to a different pH. As no recovery experiments were cited by Levan and Tjio, it is likely that roots in which c-mitosis has been described were dead at the time of sampling.

Results of experiments with low concentrations, where longer treatment was required before the cells were killed, showed that spindle formation was ultimately suppressed. The number of anaphase and telophase stages was reduced, indicating that the cells go to apparently normal daughter cells, if the spindle is already initiated at the time of treatment. After 24 hours' treatment there were no anaphase or telophase stages, but some suppressed metaphases in which centromeres had not divided. The onset of prophase was also affected as the number of cells dividing during treatment was reduced. This suggests that there is a general slowing down of the metabolism of the cell, which may be evidence for a toxic action even at the lowest concentration.

"Stickiness" of anaphase and telophase chromosomes occurred almost at once with the high concentration, but more slowly at the low concentrations. It is induced by many phenols (Levan and Tjio, 1948). Like spindle suppression, stickiness, particularly of anaphase chromosomes, results from treatment with a wide variety of organic substances and also by cold treatment (D'Amato, 1948). There is no evidence to suggest that these treatments all have the same specific action on the chromosomes.

It seems possible that the cytological abnormalities induced by nitrophenols reflect the general toxic action of these substances on the cells. Spindle suppression, stickiness of chromosomes, and granulation and pycnosis of the resting nuclei could be a direct result of the slow death of the cell. Phenols are known to precipitate proteins and the results for nitrophenols can be explained as a slow fixation process, which first affects spindle formation and then the onset of prophase, gradually slows the whole cell division process and kills the resting nuclei.

On the other hand, the nitrophenols at low concentrations may have a specific action on the spindle mechanism not at the same point as colchicine, as well as an unrelated toxic action which kills the cells before the abnormal mitosis is complete. As no abnormalities have been seen in recovered roots, it is not known whether inhibited metaphase cells found in roots after 24 hours at low concentrations undergo centromere division before reaching the resting condition. Further work is necessary at low concentrations before this possibility can be excluded.

It seems essential to make a distinction between the specific action of a chemical on one phase of the cell division mechanism and a general action which is apparent only on cells which are dividing. Colchicine has a specific action on one stage of the cell division process, whereas nitrophenols probably induce a general toxic effect by some metabolic derangement which is visible at first only in dividing cells. Some other substances described as c-mitotic may well have this general action and the literature should be re-examined from this point of view. The action later extends to resting nuclei which are killed. It is difficult to make the distinction with this type of experiment, as the course of an abnormal mitosis cannot be followed through a complete division. It is necessary to reconstruct the process from a knowledge of the normal mitosis and observations on a series of fixed roots.

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EXPLANATION OF PLATE XIII.

Effect of 2,4-dinitrophenol on Onion root tip cells. (All photographs × *ca* 1000.)

1-3. Metaphase after treatment with 3, 30 and 300 mg/l respectively.

4-5. Anaphase showing sticky bridges after 30 and 300 mg/l respectively.

6-9. Abnormal metaphase and anaphase stages after attempted recovery in water for 24 hours following treatment with 30 and 300 mg/l.